

## Physico-chemical, nutritional and microbiological potential of the pulp and juice of a wild Côte d'Ivoire fruit (*Annona senegalensis*): Effects of temperature and storage time

Mariame Cissé<sup>1</sup>, Djary Michel Koffi<sup>1</sup>, Ginette Gladys Doué<sup>1,\*</sup>, Aya Carole Bonny<sup>1</sup> and Yadé René Soro<sup>1,2</sup>

<sup>1</sup> Laboratory of Biotechnology, Agriculture and Valorization of Biological Resources, Biotechnologies UPR; UFR Biosciences; Félix Houphouët-Boigny University, Abidjan, Côte d'Ivoire.

<sup>2</sup> Laboratory of Biochemistry, Microbiology and Valorization of Agricultural Resources, Institute of Agropastoral Management, University Peleforo Gon Coulibaly, Korhogo, Côte d'Ivoire.

World Journal of Advanced Research and Reviews, 2024, 22(03), 336–349

Publication history: Received on 16 March 2024 revised on 01 June 2024; accepted on 03 June 2024

Article DOI: <https://doi.org/10.30574/wjarr.2024.22.3.1260>

### Abstract

In order to add value to wild fruits, a wild fruit tree, *Annona Senegalensis*, consumed in Côte d'Ivoire, was identified and its fruit pulp used for juice production. Biochemical, nutritional and microbiological characterizations of the pulp and of the formulated juices, pasteurized or not, were carried out, as well as the stability over time of these juices. The results indicate that *Annona senegalensis* pulp, which is acidic with a pH of 5.63 and a titratable acidity of 3.86 mEq/100g MF, has a high water content (74.03 g/100g MF), total free sugars (2679.66 mg/100g MF) and is also a good source of vitamins (vitamin C (50.28 mg) and provitamin A (106.98 µg)/100g MF), minerals with the macromineral K, Ca, Mg and P contents of 119.09, 28.6, 19.30 and 11.49 mg/100g respectively and the oligomineral Fe (mg), Na and Zn contents of 3.6, 35.90 and 0.96 µg/100g respectively in fruit pulp and phenolic compounds, with total polyphenol and flavonoid contents of 439.79 and 9.36 mg/100g MF. The juice formulated from *Annona senegalensis* pulp is also rich in its various nutrients, which have not been altered by heat treatment. On the contrary, the result is a pasteurized juice of satisfactory quality compared to the control germs of faecal contamination, with no health risk to consumption. A study of the stability of pH, free sugars, total polyphenols and vitamin C over time showed that pasteurized juices obtained from this pulp keep better at 4°C, while maintaining good microbiological quality throughout these storage days.

**Keywords:** Wild Fruit; *Annona Senegalensis*; Fruit Juice; Microbiological Analysis; Storage

### 1. Introduction

Diet is a determining factor in a healthy body [1] and nutritional recommendations have been made to encourage the adoption of a healthy diet [2] through the introduction of fruit and vegetables into our dietary habits. A number of epidemiological studies have shown that low consumption of fruit and vegetables increases the risk of chronic disease in humans. To this end, the combined report of the World Health Organisation (WHO) and the FAO on the prevention of chronic diseases recommends the consumption of at least 400 g of fruit and vegetable per person per day [3].

The importance of this type of food is due to the quantity and quality of the nutrients it contains. They provide the body with an abundance of sugars, vitamins, minerals and fiber essential for proper functioning and health [4]. In addition, fruits provide a number of phytochemicals such as carotenoids and phenolic compounds, which benefit the human body in the prevention of a wide range of chronic diseases [5]. Thus, the availability, accessibility and diversification of these fruits with a view to regular consumption appear to be essential ways and means of combating the world's growing food insecurity.

\* Corresponding author: Ginette Gladys Doué

However, alongside these commonly consumed fruits, there are thousands of wild fruits in Africa, little known and little consumed. Nevertheless, these wild fruits are used on a daily basis, not only by a few remaining hunter gatherer communities, but also by the vast majority of African farming population [6]. They make an essential contribution to the nutrition of people in these communities, ensuring their survival in times of shortage and providing essential nutrients usually lacking in the diet derived from cooked foods.

In Côte d'Ivoire, little is known about wild fruits and research into harvested fruit trees is often limited to more or less complete inventories. Many of these fruit species, although known to local farmers populations who commonly consume them, are not popularized on a national scale, which explains the lack of information about them. The reasons are organoleptic, prejudice (social status), eating habits and above all lack of information on their nutritional or even therapeutic potential [7]. A better understanding of these fruit species in relation to their composition in bioactive substances, followed by the dissemination of research results, will provide a lasting solution to the problems of food insecurity. These fruits are particularly rich in diversity, including *Annona Senegalensis* Pers, whose fruit is the second most consumed fruit in its production zone in Côte d'Ivoire.

This wild fruit, *Annona Senegalensis* Pers, could be an important source of nutrients given its vital importance in satisfying many local needs and the nutritional benefits due to the richness of its pulp in many essential nutrients. However, fruits are susceptible to spoilage due to their high water content (76%) when ripe. Spoilage is accentuated by physicochemical and enzymatic changes involving oxidation or microbial deterioration leading to fermentation [8]. These changes alter both nutritional composition and sensory attributes of the fruit, making it unfit for consumption [9]. To protect fruit from this deterioration and resulting total loss, it is particularly necessary to keep it cold or process it into fruit juice [8]. This process is an alternative to the problem of preserving highly perishable foodstuffs, which people has always had to face his survival in times of shortage. Processing will allow access to the benefits of fruit at any time, as fresh fruit is consumed during short periods of season availability after being picked from wild tress or after harvesting [10]. This study is part of an initiative to enhance and promote local wild products in Africa by characterizing the biochemical and organoleptic properties of their pulp and the juices derived from their processing. Its aim is to contribute to the nutritional enhancement of a wild fruit, *Annona Senegalensis* Pers (cinnamon apple) consumed in central and northern Côte d'Ivoire.

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## 2. Materials and Methods

### 2.1. Materials

Fruits of *Annona Senegalensis* Pers (cinnamon apple), harvested at maturity from march to june 2023 (Figure 1) in Yamoussoukro (Bélier region, 6° 48' 36" N, 5° 17' 44" W) and in Bouaké capital of the Bandama Valley region (7° 49' 60" N and 4° 55' 0" W) constituted the samples. They were stored in refrigerated, sterile coolers and transported to the Biotechnology's laboratory of UFR Biosciences at Felix Houphouët-Boigny university for analysis.



**Figure 1** Photograph of ripe cinnamon apples

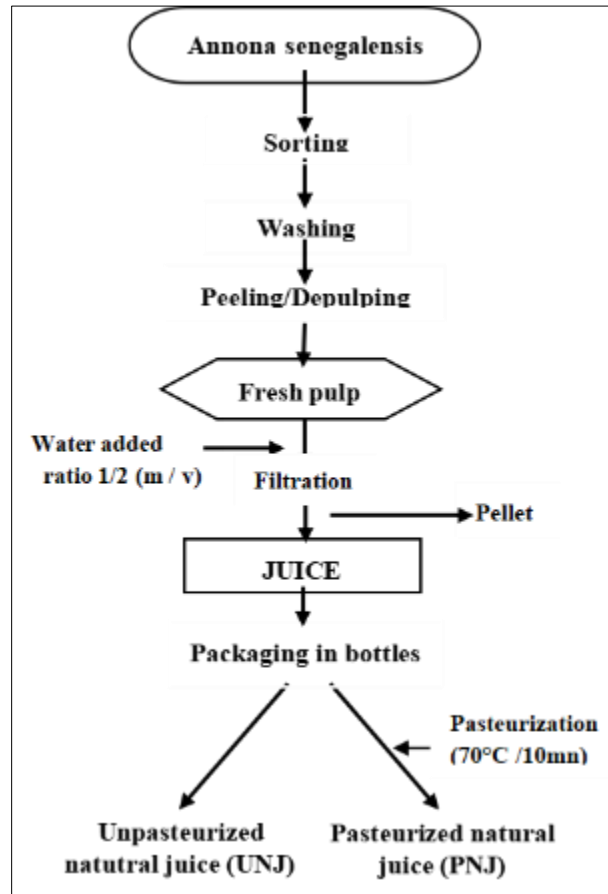
### 2.2. Methods

#### 2.2.1. Fresh pulp process

Collected samples undergo several preliminary treatments before being analyzed. The fruit was sorted to remove stems, debris and impurities (rotten fruit). The healthy fruit was then washed with tap water and drained at laboratory temperature. Finally, the clean fruits from the wash were peeled and seeded to obtain the pulp [11], which was collected in a clean, sterile glass jar, hermetically sealed and stored in the refrigerator at 4° C.

### 2.2.2. Juice production process

The transformation process described below (Figure 2) was based on the soursop juice transformation model produced by [11], with a slight modification. The fresh fruit pulp (500 g) was pureed by adding twice the volume of water. The resulting paste was filtered through muslin to extract the juice, which was collected in 250 mL sterile glass bottles. Two batches of bottles were made up: one for pasteurization and one unpasteurized.



**Figure 2** Process diagram for juice production from *Annona senegalensis* fruit [11]

### 2.2.3. Pasteurization process

Pasteurization was carried out by placing the juice bottles (250 mL) in a water bath under constant agitation. A probe was placed inside one of the juice bottles to measure the temperature. When the temperature reached 70 °C, it was maintained at this level for 10 minutes, after which the bottles were removed from the bath and left to cool to room temperature.

### 2.2.4. Juice storage process

Natural juices from both batches (pasteurized and unpasteurized) were kept refrigerated at 4 °C for 15 days. One bottle from each batch was taken out of the fridge each day and left to stand at room temperature for 10 minutes before analysis.

### 2.2.5. Proximal composition

Proximate values were evaluated using standard analytical methods. Thus, moisture, ash, crude fiber, protein, and lipid contents were obtained according to [12] standard methods. Moisture content was determined by drying the samples in a convection oven at 105 °C to constant weight.

Crude ash content was assessed by incinerating dry ground samples in a muffle furnace at 550 °C until all organic matter was removed. Crude fiber content was determined after digestion with sulfuric acid, removal of alkalis by successive washing with hot water, drying (105 °C, 8 h) and incineration of residues (550 °C, 3 h) in a muffle furnace. Crude lipid

content was determined by continuous flow and reflux extraction in a soxhlet apparatus for 7 hours using hexane as solvent. Crude protein was determined by the Kjeldahl method after digestion and distillation, and total protein content was calculated using the 6.25 factor for conversion of total nitrogen to total protein. Reducing sugar content was determined after extraction of water-soluble sugars and measurement by the DNS (dinitrosalicylic acid) method described by [13]. Total soluble sugar content was determined by the phenol-sulfuric method described by [14] after water-soluble extraction. Available carbohydrate and starch contents were calculated by difference, while energy values was calculated using conversion factors. Titratable acidity was assessed by titration of a volume of supernatant obtained after dilution of the sample in distilled water according to [15], while pH was measured using the electrode of a calibrated pH meter.

#### 2.2.6. Mineral analysis

Mineral elements such as potassium, calcium, sodium, iron, zinc and magnesium were analyzed from the ash using an atomic absorption spectrophotometer (GBC 904AA; Germany, detection limit: 1.1 ppm) [16]. In the case of phosphorus, 20 mL of demineralized water and 10 mL of vanadium molybdate reagent (0.025 mg/mL) were added to 5 mL of mineralization in a 50 mL flask and left to stand for 30 minutes after homogenization. The optical density of the mineralized sample was read at 400 nm using a spectrophotometer (Spectronic 20 D). The phosphorus concentration of the sample was determined from a calibration line established using a 0.0025 mg/mL phosphorus stock solution under the same conditions.

#### 2.2.7. Phytochemical analysis

##### Vitamin C content

The method described by [17] was used to determine the vitamin C content of the samples. After extraction with a mixture of metaphosphoric acid/acetic acid (2%; m/v), the amount of vitamin C was titrated with a 0.5 g/L solution of 2,6-DCPIP (2,6-dichlorophenol indophenol) and then calculated as a percentage of dry sample mass using the formula (1):  $Vitamin\ C(\%) = (C \times V \times fd \times 10^{-3} / me) \times 100$  (1)

C: 2,6-DCPIP solution concentration; fd: dilution factor ; me: mass (g) of pulp ; V: volume (mL) of 2,6-DCPIP at equivalence

##### $\beta$ -carotene content

$\beta$ -carotene content of extracts is given by the relationship described by [18] taking into account the  $\beta$ -carotene extinction coefficient ( $A_{1cm}^{\%}$ ) and absorbance of total carotenoids extracted in hexane. For this purpose, after saponification and hexane extraction, the optical density (OD) of the total carotenoid extracts was measuring the with a spectrophotometer (Milton ray, USA) at 450 nm. The  $\beta$ -carotene content was then determined using the extinction coefficient A and expressed in  $\mu\text{g/g}$  according to formula (2):

$$C \left( \frac{\mu\text{g}}{\text{g}} \right) = (OD \times VT \times 10^4) / (A_{1cm}^{\%} \times me) \quad (2)$$

VT is total volume of supernatant;  $A_{1cm}^{\%}$  is extinction coefficient of  $\beta$ -carotene equivalent to 2592; and me is mass of the sample (g).

##### Total polyphenol content

Total polyphenol content (TPC) was quantified by Folin-ciocalteu method [19] after extraction with methanol. A standard range established from a gallic acid stock solution (1 mg/mL), under the same conditions as the test determined the amount of polyphenol in the sample. TPC was expressed as gallic acid equivalents (mg GAE/g dry weight).

##### Total flavonoid content

Total flavonoids content was quantified by [20] method after extraction with methanol. The absorbance was measured with a spectrophotometer at 415 nm against a blank. A calibration range was performed from a 0.1 mg/mL quercetin stock solution.

### 2.2.8. Microbiological analysis

A number of germs were counted in the pulp and in various natural juices (pasteurized and unpasteurized) to assess microbiological quality control on D0 (day 0) and during the 16 days of storage.

GAM were counted on plate count agar medium (PCA, Oxoid LTD, UK); staphylococci on Baird Parker agar (Bio-Rad, France) and sulfite-reducing anaerobes on Triptone Sulfite Cycloserine (TSC) agar (BioMérieux, Franc) using standard analytical methods [21].

Neutral red bile lactose crystal violet agar (VRBL agar) was used for coliform enumeration according to the method described by [22]. Inoculation was performed by incorporation and incubation was carried out in an oven for 24 hours at 37 °C for total coliforms and 44 °C for fecal (thermotolerant) coliforms. All bright red to pink colonies with a purple halo were counted.

As for yeast and mold enumeration, it was the Yeast Extract Glucose Chloramphenicol (YGC) agar was used according to the method of [22] and salmonella detection in the samples was carried out according to the method [23], comprising four stage (pre-enrichment, enrichment, selective isolation and identification).

### 2.3. Statistical analysis

A descriptive analysis of the results was first carried out using Microsoft Office Excel 2016, to determine means and standard deviations. These means were then statistically analyzed using the ANOVA method with STATISTICA 7.5 software. The means of three tests were compared using Fisher's LSD (Least Significant Difference). This analysis method looks for means that differ significantly when  $p < 0.5$ .

## 3. Results

### 3.1. Proximate composition of fresh pulp and natural juices from *Annona senegalensis*

The proximate composition (g/100g) of the fresh pulp and natural juices of *Annona Senegalensis* fruit samples are depicted in Tables 1 and 2. For fresh pulp (Table 1), sample analysis showed pH values of  $5.63 \pm 0.11$ , titratable acidity of 3.6 meq/100g, moisture of  $74.02 \pm 0.07\%$ , and fiber of  $5.16 \pm 0.05$ . As concern ash, proteins and lipids the values obtained are  $1.40 \pm 0.12$ ;  $0.76 \pm 0.15$  and  $1.84 \pm 1.23$ . Similarly, the carbohydrates content assessed in the pulp of this fruit of  $16.50 \pm 0.75$  is shared between starch ( $12.43 \pm 0.51\%$ ), total free sugars ( $2679.66 \pm 0.07$  mg) of which  $600.65 \pm 2.16$  mg are reducing sugars while this fruit has an Energy Value of  $85.60 \pm 0.36$  Kcal/100 g.

**Table 1** Proximate composition of fresh pulpe (100 g) from *Annona senegalensis*

Parameters	Fresh pulp
pH	$05.63 \pm 0.11$
Titratable acidity (meq)	$03.86 \pm 0.07$
Moisture (g)	$74.02 \pm 0.07$
Fibers (g)	$05.16 \pm 0.05$
Ash (g)	$01.40 \pm 0.12$
Proteins (g)	$00.76 \pm 0.15$
Lipids (g)	$01.84 \pm 1.23$
Reducing sugars (mg)	$600.65 \pm 2.16$
Total sugars (mg)	$2679.66 \pm 0.07$
Carbohydrates (g)	$16.50 \pm 0.75$
Starch (g)	$12.43 \pm 0.51$
Energy value (Kcal)	$85.60 \pm 0.36$

Values are average of three tests, affective by standard deviations

On the other hand, no significant difference was observed between pasteurized and unpasteurized natural juices derived from *Annona Senegalensis* pulp (table 2). Thus, unpasteurized natural juice showed values for pH ( $5.48 \pm 0.01$ ), titratable acidity ( $0.88 \pm 0.19$  meq/100g), ash ( $0.13 \pm 0.05\%$ ), total sugars ( $393.24 \pm 2.59$  mg/100g) and reducing sugars ( $215.26 \pm 5.09$ ) significantly similar to those of pasteurized natural juice with  $5.50 \pm 0.05$  (pH),  $1.00 \pm 0.00$  meq/100g (titratable acidity),  $0.13 \pm 0.05\%$  (ash),  $211.86 \pm 5.23$  mg/100g (reducing sugars) and  $394.72 \pm 1.41$  mg/100g for total sugars.

**Table 2** Proximate composition of different natural juices made from *Annona Senegalensis*

	UNJ	PNJ
pH	$5.48 \pm 0.01^a$	$5.50 \pm 0.05^a$
Titratable acidity (meq)	$0.88 \pm 0.19^a$	$1.00 \pm 0.00^a$
Humidity (g)	$88.13 \pm 0.00^a$	$88.13 \pm 0.01^a$
Dry matter (g)	$11.87 \pm 0.19^a$	$11.87 \pm 0.23^a$
Ashes (g)	$0.13 \pm 0.05^a$	$0.13 \pm 0.05^a$
Reducing sugars (mg)	$215.26 \pm 5.09^a$	$211.86 \pm 5.23^a$
Total sugars (mg)	$393.24 \pm 2.59^a$	$394.72 \pm 1.41^a$

Values are average of three tests  $\pm$  standard deviation. The statistical differences between averages at 95% confidence are indicated on the same column by different letters. UNJ : unpasteurized natural juice, NPJ : natural pasteurized juice

### 3.2. Phytochemical composition of fresh pulp and natural juices from *Annona senegalensis*

Table 3 reported the results concerning the main phytochemical composition (100 g) obtained from the fresh pulp and the various natural juices made from *Annona Senegalensis*. Statistical analysis of these results revealed significant differences ( $P < 0.05$ ) between these different samples with regard to the studied parameters. Overall, the pulp's vitamin C ( $50.28 \pm 0.07$ ), polyphenol ( $439.79 \pm 13.22$  mg), flavonoid ( $9.36 \pm 0.73$  mg) and  $\beta$ -carotene ( $106.98 \pm 1.76$   $\mu$ g) contents were far higher than those of the two natural juices, pasteurized and unpasteurized. On the other hand, these two juices showed statistically identical contents for these same parameters.

**Table 3** Main phytochemical composition (100 g) of the fresh pulp and different natural juices from *Annona Senegalensis*

	Fresh pulp	UNJ	NPJ
Vitamin C (mg)	$50.28 \pm 0.07^a$	$40.66 \pm 0.57^b$	$39.66 \pm 0.47^b$
TPC (mg)	$439.79 \pm 13.22^a$	$87.73 \pm 0.29^b$	$85.73 \pm 0.19^b$
TFC (mg)	$09.36 \pm 0.73^a$	$05.23 \pm 0.29^b$	$06.23 \pm 0.22^b$
$\beta$ -carotene ( $\mu$ g)	$106.98 \pm 1.76^a$	$82.90 \pm 0.72^b$	$81.90 \pm 0.62^b$

The values in the table are averages of three tests, assigned standard deviations. Statistical differences in juice and fresh pulp between these averages at 95% confidence level are indicated on the same line by the different letters. TPC: total polyphenol content; TFC: total flavonoid content. UNJ : unpasteurized natural juice, NPJ : natural pasteurized juice

### 3.3. Mineral content

The results concerning the main studied macro and oligo minerals of the pulp and juices have been consigned in the Table 4. Minerals contents in the fresh pulp samples were significantly different from that of juices at  $p < 0.05$ .

The results showed that the macromineral K, Ca, Mg and P contents of 119.09, 28.6, 19.30 and 11.49 mg/100g respectively (Table V) and the oligomineral Fe (mg), Na and Zn contents of 3.6, 35.90 and 0.96  $\mu$ g/100g respectively in fruit pulp were higher than those found in unpasteurized and pasteurized natural juices, which were significantly identical.

**Table 4** Mineral composition of fresh pulp and pasteurized and non pasteurized juices from *Annona Senegalensis* (100g)

	Fresh pulp	UNJ	NPJ
P (mg)	11.49±1.00 <sup>a</sup>	7.8±0.85 <sup>b</sup>	7.8±0.85 <sup>b</sup>
K (mg)	119.09±0.02 <sup>a</sup>	103.8±8.80 <sup>b</sup>	103.8±8.80 <sup>b</sup>
Ca (mg)	28.60±2.45 <sup>a</sup>	24.76±2.05 <sup>b</sup>	24.76±2.05 <sup>b</sup>
Mg (mg)	19.30±1.69 <sup>a</sup>	17.93±1.54 <sup>b</sup>	17.93±1.54 <sup>b</sup>
Fe (mg)	3.60±0.10 <sup>a</sup>	0.67±0.05 <sup>b</sup>	0.67±0.05 <sup>b</sup>
Na (µg)	35.90±3.11 <sup>a</sup>	11.66±1.15 <sup>b</sup>	11.66±1.15 <sup>b</sup>
Zn (µg)	0.96±0.08 <sup>a</sup>	0.75±0.06 <sup>b</sup>	0.75±0.06 <sup>b</sup>

The values in the table are averages of three tests, assigned standard deviations. The statistical differences between these averages at 95% confidence level are indicated on the same line by the different letters. UNJ: unpasteurized juice NPJ: natural pasteurized juice

### 3.4. Microbiological quality analysis of pulp and juice

Table 5 depicted the quality control results for *Annona senegalensis* pulp and juices. Of all the germs counted and/or tested, sulphite-reducing anaerobes (SRA), salmonella, total and thermotolerant coliforms were totally absent, as were *E. coli* and *Staphylococcus aureus*.

**Table 5** Microbial load of pulp and pasteurized and non pasteurized juices from *Annona Senegalensis*

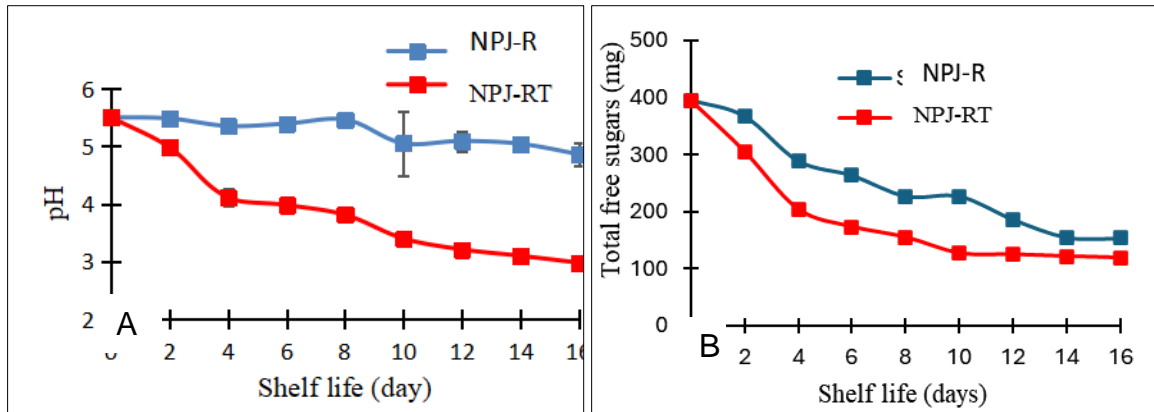
	pulp (CFU/mL)	UNJ (CFU/mL)	NPJ (CFU/mL)	CODEX 247-2005
GAM	150	108	20	< 10 <sup>3</sup> cfu/mL
Col T	00	00	00	< 10 cfu/mL
col Ther	00	00	00	< 1 cfu/mL
Y/M	105	76	23	Y:<200cfu/mL M<100cfu/mL
A S R	00	00	00	none/mL
S A	00	00	00	none/mL
Sal	none	none	none	none/25mL

Cfu: colony-forming unit; GAM: mesophilic aerobic germ; Sal: salmonella; S A: staphylococcus aureus; A S R : sulfite-reducing anaerobes ; Col T: total coliforms; col Ther: thermoresistant coliforms and Y/M: yeast/mold. UNJ : unpasteurized natural juice, NPJ : natural pasteurized juice

On the other hand, in regard to GAM and yeast/molds, the number of germs counted in the pulp (150 cfu/mL) dropped steadily in the unpasteurized plain juice (108 cfu/mL) and then in the pasteurized plain juice (20 cfu/mL).

### 3.5. Monitoring the main parameters of pasteurized natural juices stored at room temperature and refrigerator

The results of the pH variation of pasteurized natural juice samples stored at room temperature and in the refrigerator are shown in Figure 2 A. Analysis of these results reveals a steady fall in pH, which is less pronounced with a drop of 11.63% (5.5 to 4.86 for refrigerated storage) and a more significant drop of around 45.81% (5.5 to 2.98 for storage at room temperature) over the 16 days of storage.

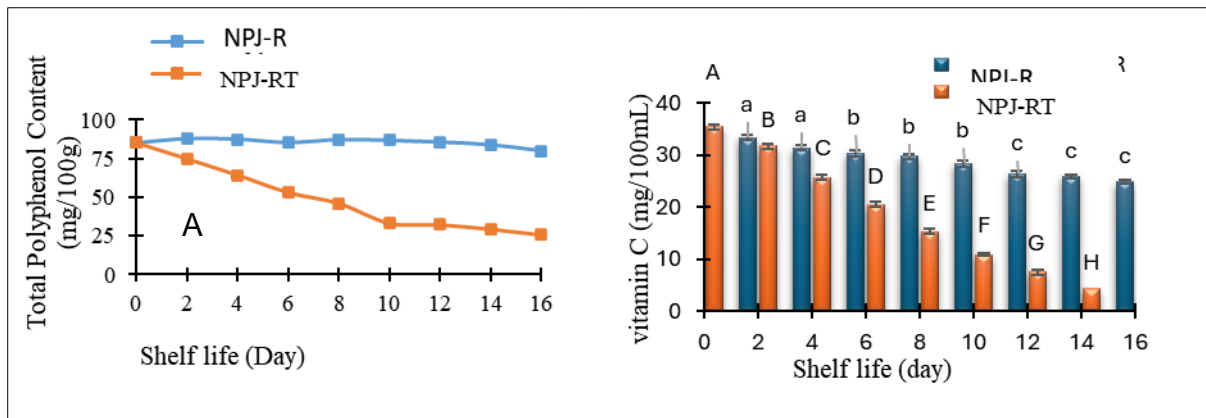


NPJ-R: natural pasteurized juice stored at refrigerator; NPJ-RT: natural pasteurized juice stored at room temperature

**Figure 3** Evolution of pH (A) and total free sugars (B) of pasteurized natural juice as a function of storage temperature: room temperature (28 °C) and refrigerator (4 °C).

As for pH, the amount of total free sugars fell in samples of pasteurized natural juice, whatever the storage method. Similarly, this drop was more pronounced (69.91%) at room temperature, where it fell from 394.72 mg/100g at Day 0 to 118.77 mg/100g at Day 16.

Figure 4 (A) displayed results relating to changes in total polyphenol content and figure 4 (B) showed the evolution of vitamin C over 16 days in samples of pasteurized natural juices as a function of storage method.



**Figure 4** Evolution of total polyphenol content (A) and vitamin C (B) of pasteurized natural juice as a function of storage temperature: room temperature (28 °C) and refrigerator (4 °C).

NPJ-R: natural pasteurized juice stored at refrigerator; NPJ-RT: natural pasteurized juice stored at room temperature

In this case, the amount of total polyphenols fell more rapidly by over 50%, i.e. by 69.86%, and significantly when the pasteurized juice was stored at room temperature (from 85.09 to 25.66 mg/100g after 16 days), whereas the reduction was more moderate at 5.86% (from 85.09 to 80.1 mg/100g) for pasteurized juice stored in the refrigerator.

The vitamin C content of juice stored at room temperature (NPJ-RT) dropped drastically by more than 85% (87.74%), from 35.33 to 4.33%, in contrast to juice stored in the refrigerator (NPJ-R), where the drop was relatively small, barely 30% (29.72%), from 33.3 to 24.83% over the 16 days of storage.

The results of microbiological quality control on pasteurized juice samples stored in the refrigerator are presented in Table 5 below.



**Table 5** Evolution of the microbial load of refrigerated pasteurized juices during 21 days of storage

	GAM	Col T	Y/M	ASR	SA	Sal
D0+7(log cfu/mL)	1.84	1.60	2.2	00	00	none
D0+14(logcfu/mL)	1.53	0.39	1.3	00	00	none
D0+21(logcfu/mL)	01	00	01	00	00	none

Cfu: colony-forming unit; GAM: mesophilic aerobic germ; Sal: salmonella; SA: staphylococcus aureus; ASR : sulfite-reducing anaerobes ; Col T: total coliforms; col Ther:thermoresistant coliforms and Y/M:yeast/mold; D0: day 0

Enumeration results for GAM, total coliform, yeast/mold, salmonella, streptococcus aureus and sulfite-reducing anaerobes in pasteurized juice complied with CODEX 247-2005 (Table 5). No salmonella, staphylococcus aureus or sulfite-reducing anaerobe germs were detected during 21 days of cold storage. On the other hand, loads of 01 for GAM, of 0.39 for total coliforms and 01 for yeast/mold were detected in pasteurized juice after 21 days of storage (D0+21).

#### 4. Discussion

Assessing a food's nutritional parameters is crucial to understanding its potential to meet the body's needs, and is a necessary step in the promotion of foods, particularly wild fruits.

Thus, in the case of proximal composition, freshly harvested *Anona Senegalensis* pulp showed a water content comparable to that of many fruits, including Tanzanian and Nigerian soursop fruits (76.2 and 77.8%) reported by [24, 25] and *Annona Senegalensis* fruits (74.02%) harvested in V-Baoulé in Côte d'Ivoire by [26]. Hence, due to their high water content, consumption of *Annona Senegalensis* fruits could contribute to the body's hydration and thus facilitate the supply of various nutrients to the organism. Furthermore, moisture is an important parameter for fruit quality, as it significantly affects texture, taste and microbial growth. This has a negative impact on the shelf life of these foods, which need to be quickly consumed or processed into a form that is easy to store.

pH is an important physical parameter that is inversely correlated with acidity, making it possible to assess a food's acidity level. The pH values obtained for the pulp (5.63) and the natural pasteurized and unpasteurized juices (5.48-5.50) were comparable to those of the fruit usually consumed, namely soursop pulp and juice (5.2) [27], and even higher than the pH values (3.76) obtained for cashew juice produced in Côte d'Ivoire [28]. Thus, these pH values of *annona Senegalensis* pulp and juice around 5.5, highlighting their average acidity level, could contribute to a slightly acid taste much sought-after by consumers. In fact, acidity in fruit plays an important role in the taste, color and microbial stability of fruit juice.

In general, fruits contain little fat and protein, suggesting that they are not good sources of energy. However, the average lipid content of *annona Senegalensis* fruit obtained in this study is higher than the value of 0.31 g / 100 g MF for soursop fruit, as reported by [29] and 0.22 g / 100 g MF for Tanzanian mangoes. Given its low fat content, *annona Senegalensis* could be recommended in diets aimed at preventing overweight or obesity, diabetes and hypertension [30, 31].

Assessing the amount of free sugars present in foods is essential, as excess sugar increases the risk of caries, promotes weight gain and has been incriminated in the onset and/or complications associated with type 2 diabetes and cardiovascular disease [32, 33]. In this context, the content of total free sugars observed in the pulp and in the various unpasteurized and pasteurized natural juices of *annona Senegalensis* was well below the required standards for all segments of the population: children aged 4 to 6 (<19 g/day), 7 to 10 (<24 g/day) and adults (<30 g/day), which argues in favor of consuming this fruit to help prevent the onset of these metabolic diseases.

Fruits play an important role in a balanced diet [34], thanks to their high content of micronutrients, mainly minerals. Minerals are important for maintaining the body's health, whether they are essential in large quantities (>100 mg/day, macrominerals) or in small quantities (<100 mg/day, microminerals) [35]. The values obtained in this study both for pulp and various natural juices (pasteurized or not) were higher than those recorded by [36] in the pulp of five Chilean apple cultivars, particularly with regard to K (6.8-10.70 mg/100g), Ca (4.0-4.7 mg/100g), Mg (6.8-6.3 mg/100g) as well as Fe (0.24-0.55 mg/100g) and Zn (0.00-0.03 mg/100g). This result highlights the very high micronutrient contents of the pulp of this neglected fruit, which were maintained in juices made from its pulp. Furthermore, the Fe and Cu contents of *annona senegalensis* pulp and juices, as well as the Zn content, exceed the FAO/WHO Recommended Dietary Allowances (RDAs) [37].

Calcium is one of the body's most essential macro-minerals for strong teeth and bones, and its deficiency is more widespread than that of any other mineral. Calcium and phosphorus help reduce/eradicate rickets in children and osteomalacia and osteoporosis in the elderly [38]. Magnesium functions as an activator of ATP-requiring enzymes such as hexokinase, alkaline phosphatase, fructokinase and adenylyclase. It plays a vital role in the structure and function of the human body, such as the skeleton and muscles [39]. Adult humans require around 25 mg of Mg for normal physiological functions; therefore, the levels recorded in the present study would meet safety requirements. Potassium is an intercellular salt that can combine with sodium to influence osmotic pressure and contribute to the body's normal pH balance. The relatively low levels of Na in all the fruit pulps and juices studied may reduce the incidence of hypertension, as its absorption is considered an important factor in the etiology of hypertension [38]. Zinc is involved in the normal functioning of the immune system and is associated with protein metabolism [40]. Consequently, the levels recorded in the present samples (pulp and natural juices) would contribute positively to the immune system function and protein metabolism of consumers. Iron is an essential trace element for haemoglobin formation, normal functioning of the central nervous system and oxidation of carbohydrates and fats [41].

It is also important to investigate the presence of certain phytochemicals that are known to have a positive impact on consumer health. Vitamin C and carotenoids are two important nutrient parameters determined in this study. The vitamin C contents was determined, although lower than those (77%) of orange obtained by [42], were above the daily intake recommended by the [43], for infants aged 0 to 12 months at 20 mg, for children aged 1 to 10 years at 20 to 30 mg and for pregnant and breast-feeding women at 50 mg. Consumption of *annona Senegalensis* pulp and juice could therefore help cover daily requirements of vitamin C, deficiency of which can lead to scurvy and skin haemorrhaging [44]. In addition, vitamin C acts as an antioxidant and aids iron absorption [45].

Another compound essential for healthy body function is  $\beta$ -carotene (provitamin A). For this parameter, the highest levels obtained in pulp can more than cover the daily vitamin A requirements of infants up to 12 months (350  $\mu$ g of retinol equivalent), children aged 1 to 18 (400 to 600  $\mu$ g of retinol equivalent) and pregnant and breast-feeding women (600 and 850  $\mu$ g of retinol equivalent) [3]. On the other hand, the reduction in  $\beta$ -carotene content observed in JNP juice can be explained by the thermal effect applied to it by pasteurization. As a result, the consumption of this fruit and of unpasteurized or unpasteurized plain juices can be recommended to populations for the prevention of certain diseases linked to vitamin A deficiency, notably blindness [46].

With regard to phenolic compounds, polyphenol and flavonoid levels were generally higher than those observed by [47] for pear and peach flesh respectively (210mg/100g 240mg/100g MF) for polyphenols and by [48] in apricot fruit grown in Algeria (10.01mg/100) for flavonoids. Polyphenols, by virtue of their antioxidant action, are molecules that reduce or prevent the oxidation of other substances, such as lipids [49]. As a result, this fruit and its juices could be recommended in people's diets to combat the action of free radicals, which are responsible for cardiovascular disease and cancer.

#### 4.1. Stability test

There were no significant changes in pH of pasteurized *annona Senegalensis* juices stored at refrigeration temperature up to 16 days. This means that there was no deterioration in product quality, which on the contrary was maintained. The higher pH during the first week of storage would have prevented the proliferation of pathogenic and spoilage microorganisms in the juice. A slight decrease in pH was also reported by [50] on jam prepared from different mango varieties.

Pasteurized juices in both storage modes (room temperature and refrigerator) were less stable in terms of free sugar content (showing a significant decrease from the 4th day of storage). This finding differs from that of [51], who reported an increase in free sugars in strawberry jam, and the reason for these differences may be the addition of citric acid to their jam, which must have inhibited microbial growth in the product. The decrease in free sugar during storage in this study could be due to the acid hydrolysis of polysaccharides, particularly gum and pectin [52].

Total polyphenol levels in pasteurized juice samples stored in the refrigerator were significantly high, and remained virtually constant throughout the 16-day storage period. These high levels are likely to be beneficial to consumer health. Phenols are one of the main groups of non-nutritive food components that have been associated with the inhibition of cancer, atherosclerosis and age-related degenerative brain disorders [53, 54]. These authors also reported that polyphenolic compounds have inhibitory effects on mutagenesis in humans when up to 1.0 mg is ingested daily as part of a diet rich in fruit and vegetables.

Cold-preserved pasteurized annona *Senegalensis* juices were more stable in terms of vitamin C content over the 16-day storage period, in contrast to those stored at room temperature, where a loss of over 80% was observed.

Vitamin C plays a number of antioxidant roles and helps strengthen the immune system [55]. The highest value recorded for *Annona* juice stored in the refrigerator (NPJ-R) suggests that it has the potential to deliver the health-promoting properties of vitamin C over other samples stored at room temperature. Vitamin C also contributes to food stability and appearance [56, 57]. Cold storage therefore enables better stabilization of the juice and preserves its freshness.

The pasteurization method used considerably reduced all these micro-organisms (GAM and yeast/mold), but did not eliminate them entirely. However, the relative levels were well below the recommended threshold values, testifying to the safety of the juices and the effectiveness of the heat treatment applied. Furthermore, the absence of coliforms in both pasteurized and unpasteurized juices indicated that equipment cleaning methods were satisfactory. Moreover, their absence throughout the 21-day shelf-life also testified to the effectiveness of the heat treatment applied and also indicated that juices from this local fruit may have a long shelf-life beyond 21 days.

The presence of these strains (GAM, yeast and mold) during cold storage, certainly at relatively low levels, beyond the 21-day shelf-life, could be due to the low pH of the products [58]. The presence of these organisms though in low amount need to be controlled to prevent spoilage and food borne illness [59, 60].

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## 5. Conclusion

This study highlighted the biochemical composition and nutritional potential of *Annona Senegalensis*, a neglected fruit pulp, which is widely distributed in the central region of the country. The pulp of this fruit is characterized by its acidity, richness in water, free sugars, vitamins C and A and mineral elements, making it an excellent source for a satisfactory, balanced diet. It also contains phenolic compounds such as flavonoids. A study of the stability of pH, free sugars, total polyphenols and vitamin C over time shows that pasteurized juices made from this pulp keep better at 4°C, while maintaining good microbiological quality throughout the 21 days.

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## Compliance with ethical standards

### *Acknowledgments*

The authors are grateful to the technician from the Biotechnology, Agriculture and Biological Resources Development Laboratory for technical assistances.

### *Disclosure of conflict of interest*

The authors declare no conflicts of interest.

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